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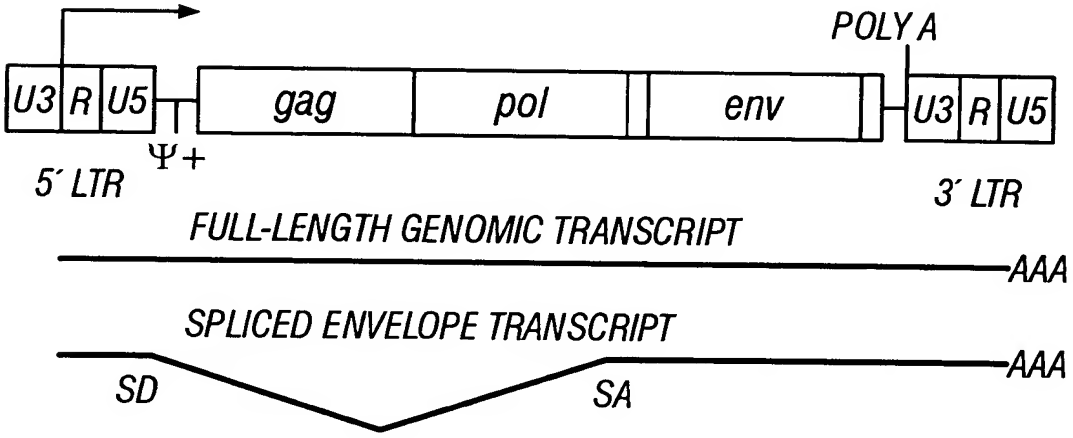
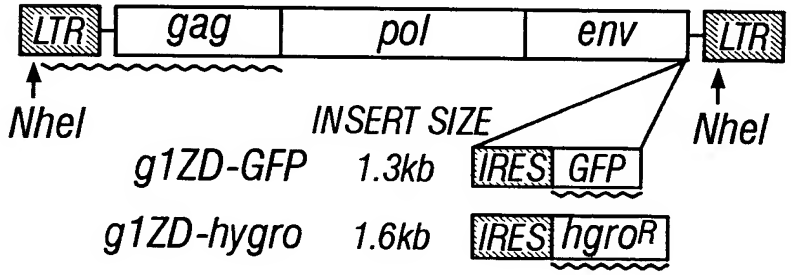


FIG. 1A



g1ZD-GFP

env IRES
...AGAGTACGAGCCA**TAG**ATAACGTTACTGGCC...
IRES GFP
...CACGATAATACC**ATG**GCCATTCGAACAAAGG...
GFP MLV NONCODING
...CTGTACAAG**TAG**CGGCCGCGCCATAGATAAA...

g1ZD-hygro

env IRES
...AGAGTACGAGCCA**TAG**ATAACGTTACTGGCC...
IRES hygromycin R
...CACGATAATACC**ATG**GCCATTCGAACCGCGA...
hygromycin R MLV NONCODING
...TGGGGGCCGCGCCA**TAG**ATAAAATAAAAGAT...

FIG. 1B

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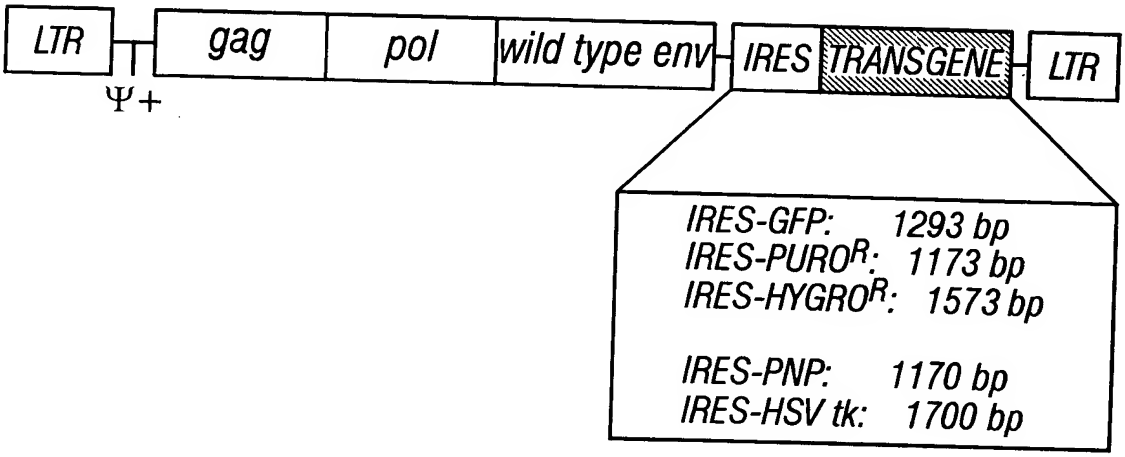

FIG. 2A

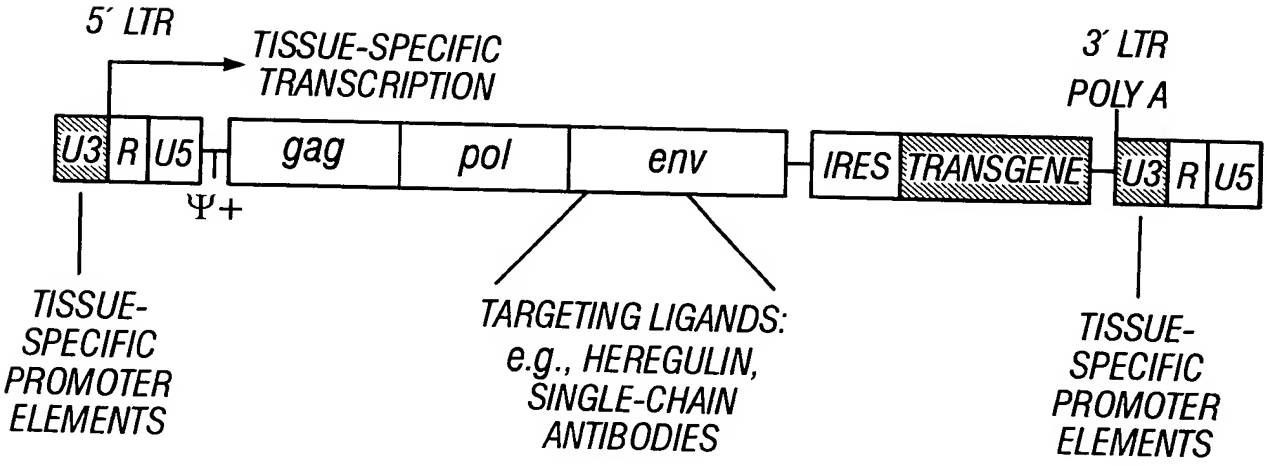

FIG. 2B

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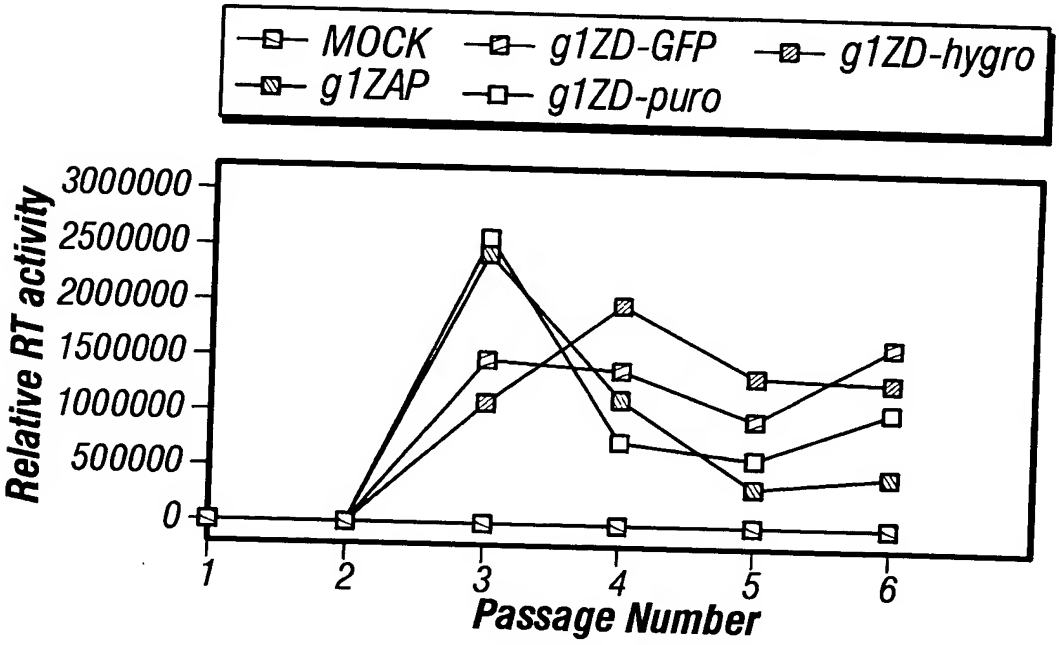

FIG. 3A

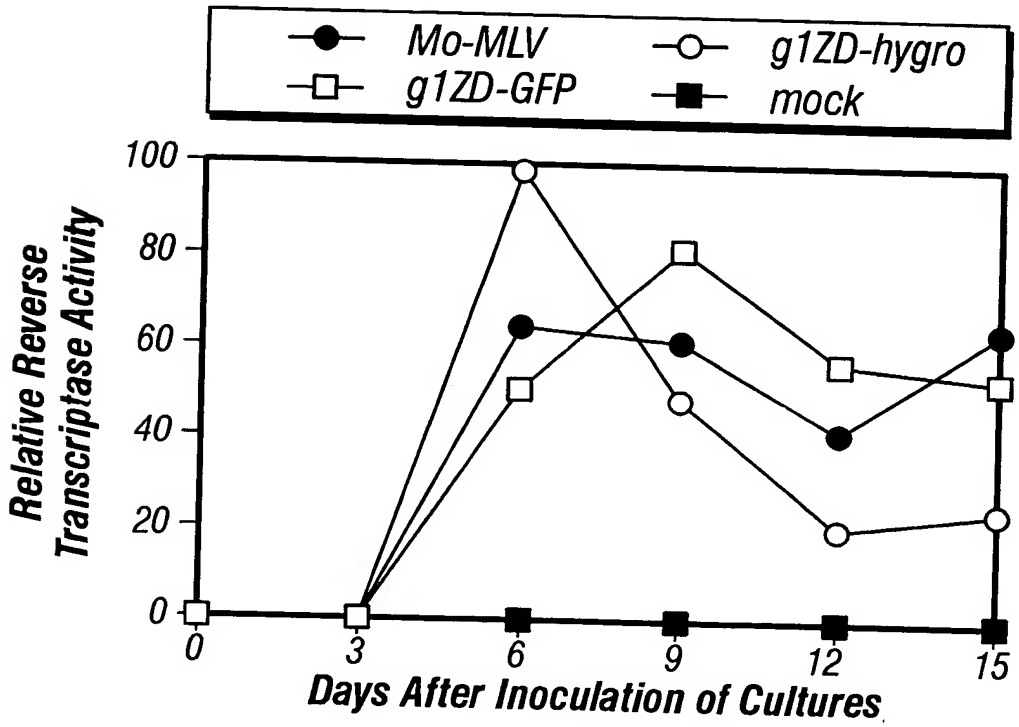

FIG. 3B

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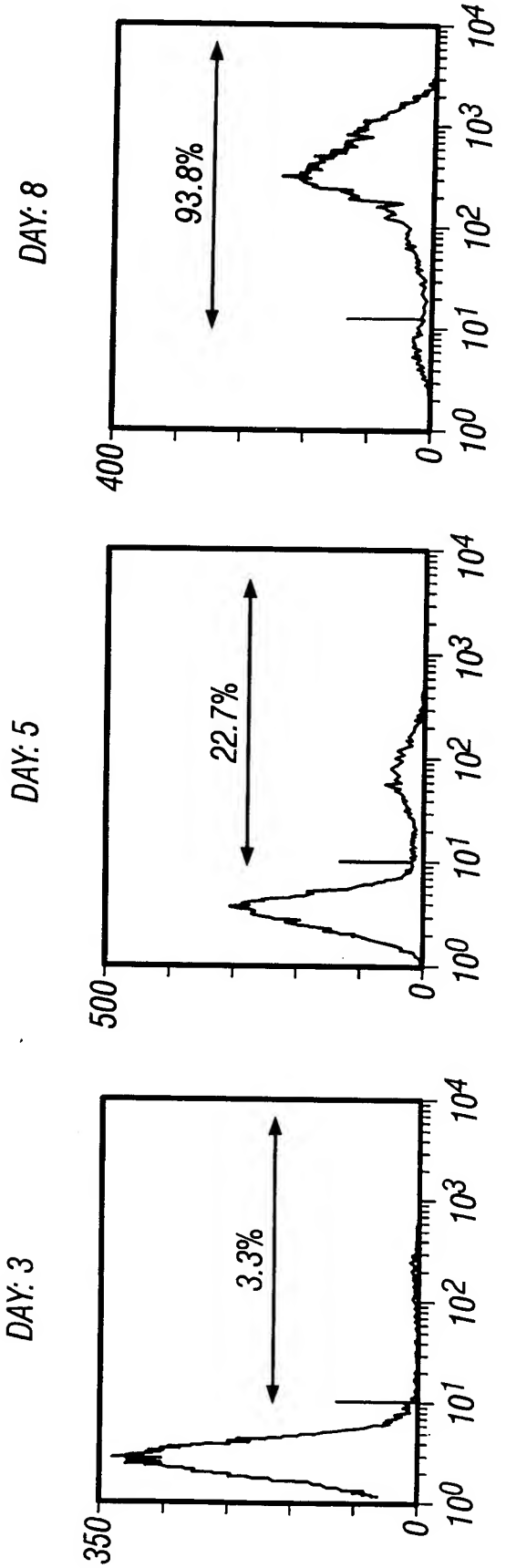
DAY: 3
3.3%
350
0
10^0
10^1
10^2
10^3
10^4
FL1
DAY: 5
22.7%
500
0
10^0
10^1
10^2
10^3
10^4
FL1
DAY: 8
93.8%
400
0
10^0
10^1
10^2
10^3
10^4
FL1

FIG. 4

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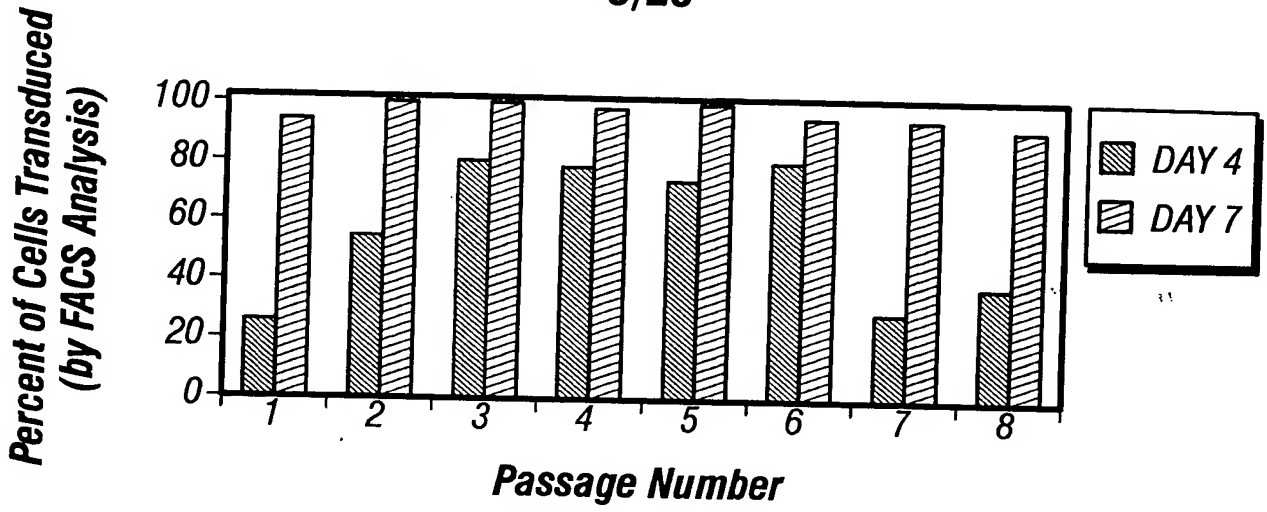


FIG. 5

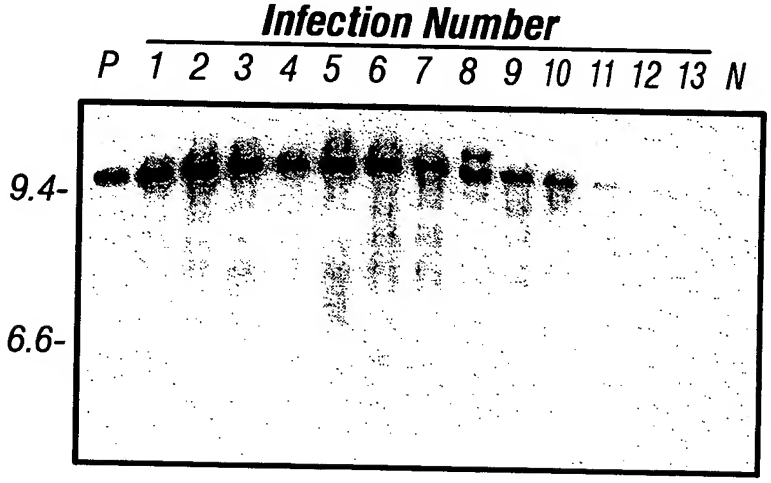
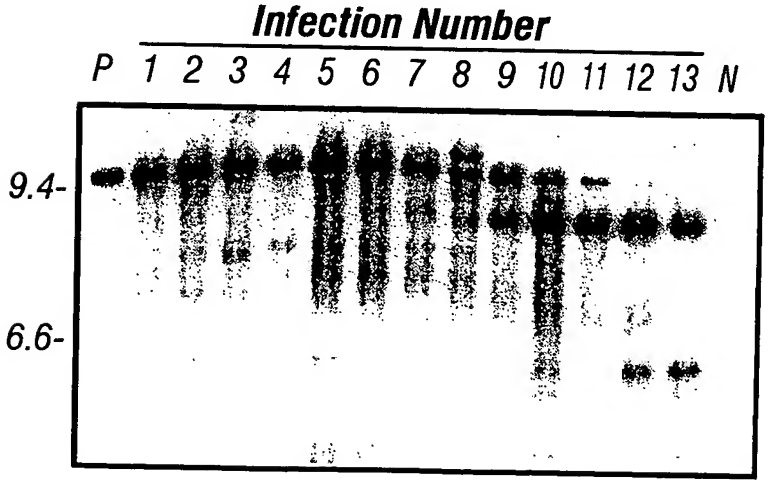


FIG. 6A



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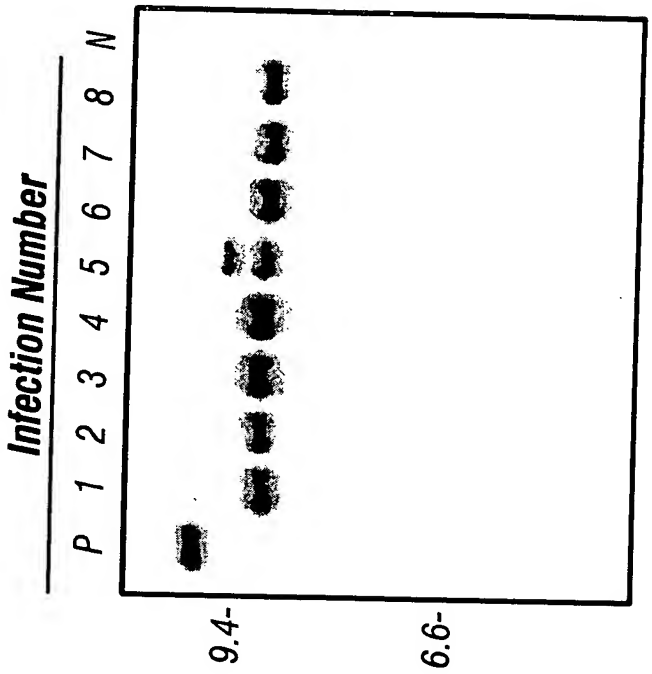
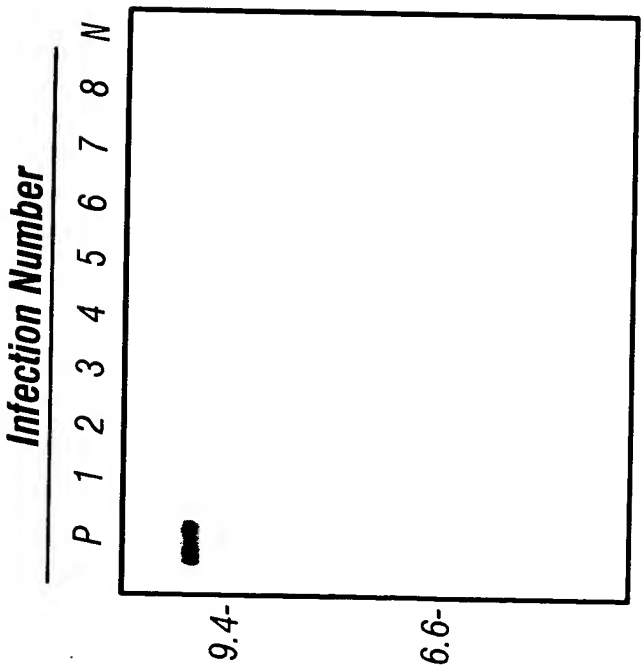

Infection Number
P 1 2 3 4 5 6 7 8 N
9.4-
6.6-

FIG. 6B


Infection Number
P 1 2 3 4 5 6 7 8 N
9.4-
6.6-

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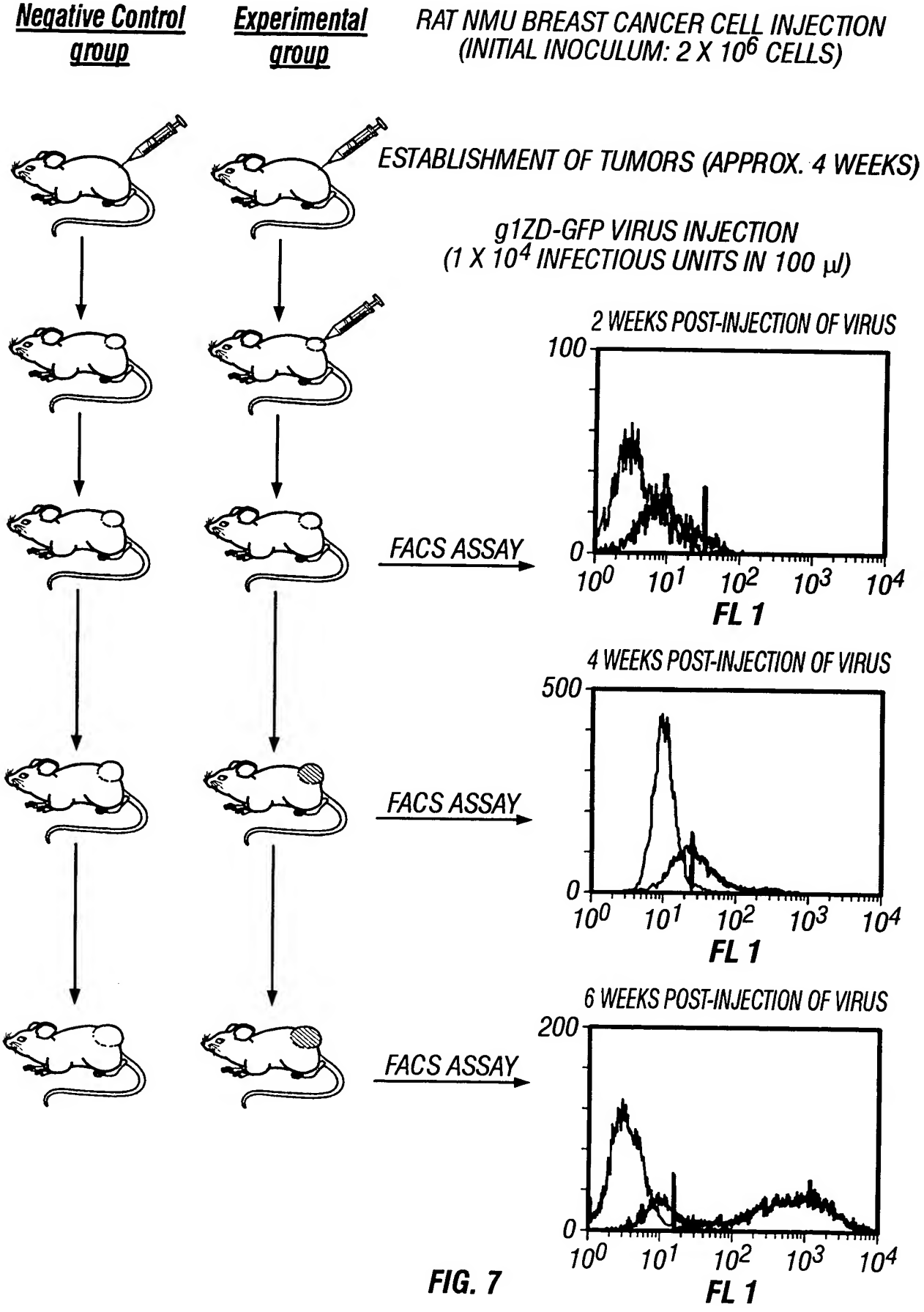
Negative Control group
Experimental group
RAT NMU BREAST CANCER CELL INJECTION (INITIAL INOCULUM: 2 X 10^6 CELLS)
ESTABLISHMENT OF TUMORS (APPROX. 4 WEEKS)
g1ZD-GFP VIRUS INJECTION (1 X 10^4 INFECTIOUS UNITS IN 100 µl)
FACS ASSAY
2 WEEKS POST-INJECTION OF VIRUS
100
0
10^0 10^1 10^2 10^3 10^4
FL 1
FACS ASSAY
4 WEEKS POST-INJECTION OF VIRUS
500
0
10^0 10^1 10^2 10^3 10^4
FL 1
FACS ASSAY
6 WEEKS POST-INJECTION OF VIRUS
200
0
10^0 10^1 10^2 10^3 10^4
FL 1

FIG. 7

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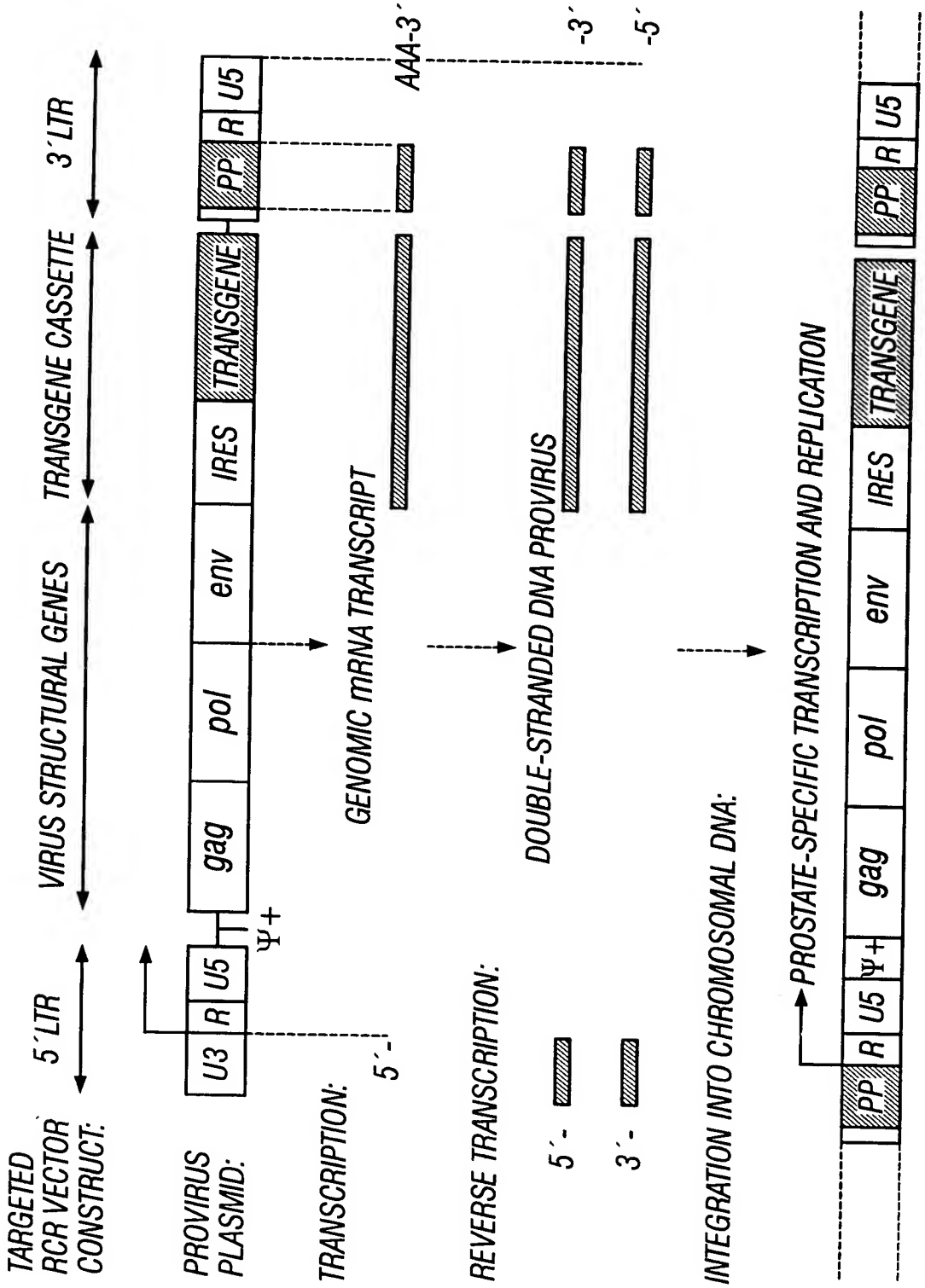
TARGETED RCR VECTOR CONSTRUCT:
5´LTR
VIRUS STRUCTURAL GENES
TRANSGENE CASSETTE
3´LTR
U3
R
U5
Ψ+
gag
pol
env
IRES
TRANSGENE
PP
R
U5
PROVIRUS PLASMID:
TRANSCRIPTION:
5´-
AAA-3´
GENOMIC mRNA TRANSCRIPT
REVERSE TRANSCRIPTION:
DOUBLE-STRANDED DNA PROVIRUS
5´-
3´-
-3´
-5´
INTEGRATION INTO CHROMOSOMAL DNA:
PROSTATE-SPECIFIC TRANSCRIPTION AND REPLICATION

FIG. 8

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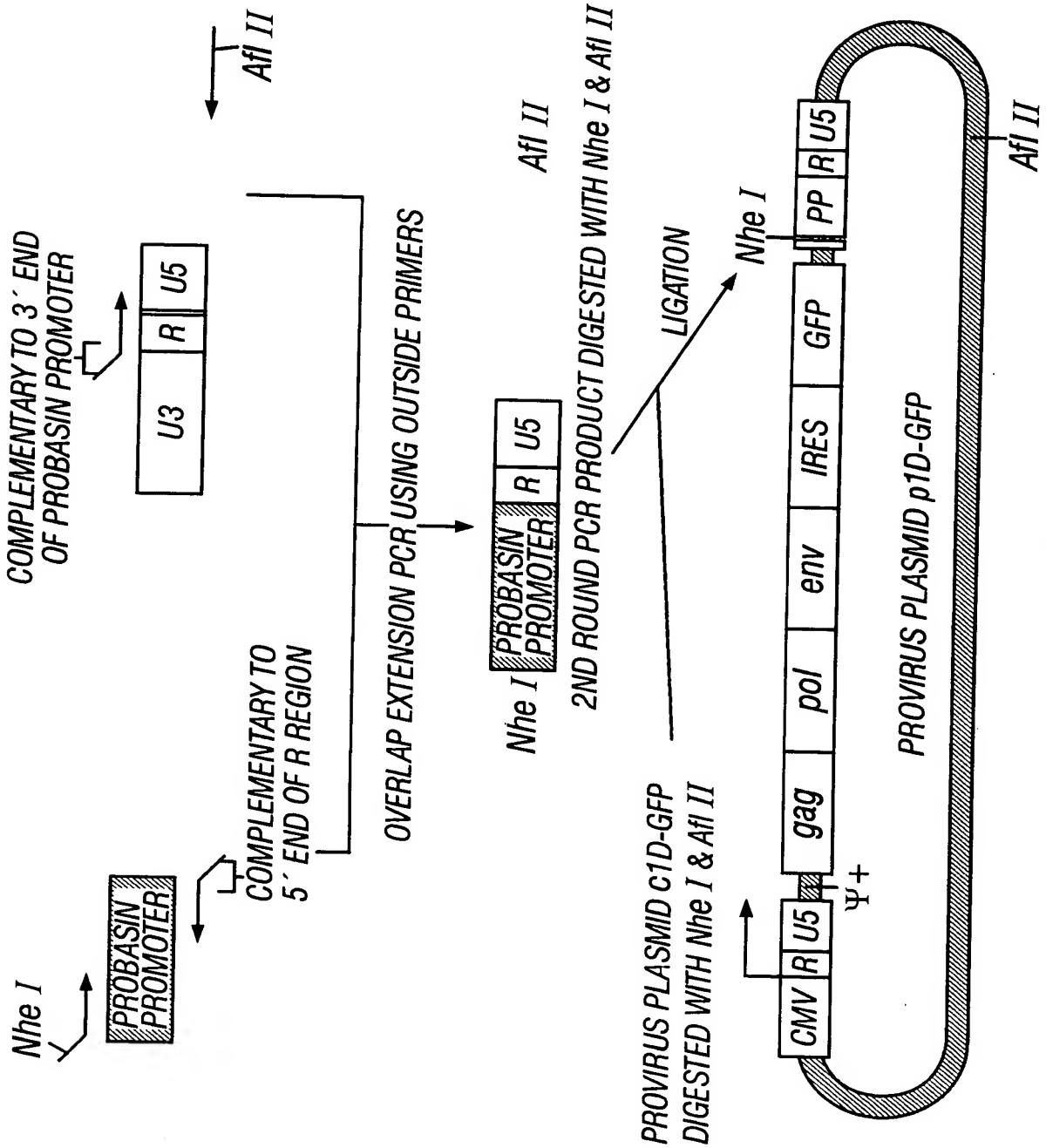
Nhe I
PROBASIN PROMOTER
COMPLEMENTARY TO 5´ END OF R REGION
COMPLEMENTARY TO 3´ END OF PROBASIN PROMOTER
U3
R
U5
Afl II
OVERLAP EXTENSION PCR USING OUTSIDE PRIMERS
Nhe I
PROBASIN PROMOTER
R
U5
Afl II
2ND ROUND PCR PRODUCT DIGESTED WITH Nhe I & Afl II
LIGATION
PROVIRUS PLASMID c1D-GFP DIGESTED WITH Nhe I & Afl II
CMV
R
U5
Ψ+
gag
pol
env
IRES
GFP
PP
R
U5
Nhe I
Afl II
PROVIRUS PLASMID p1D-GFP

FIG. 9A

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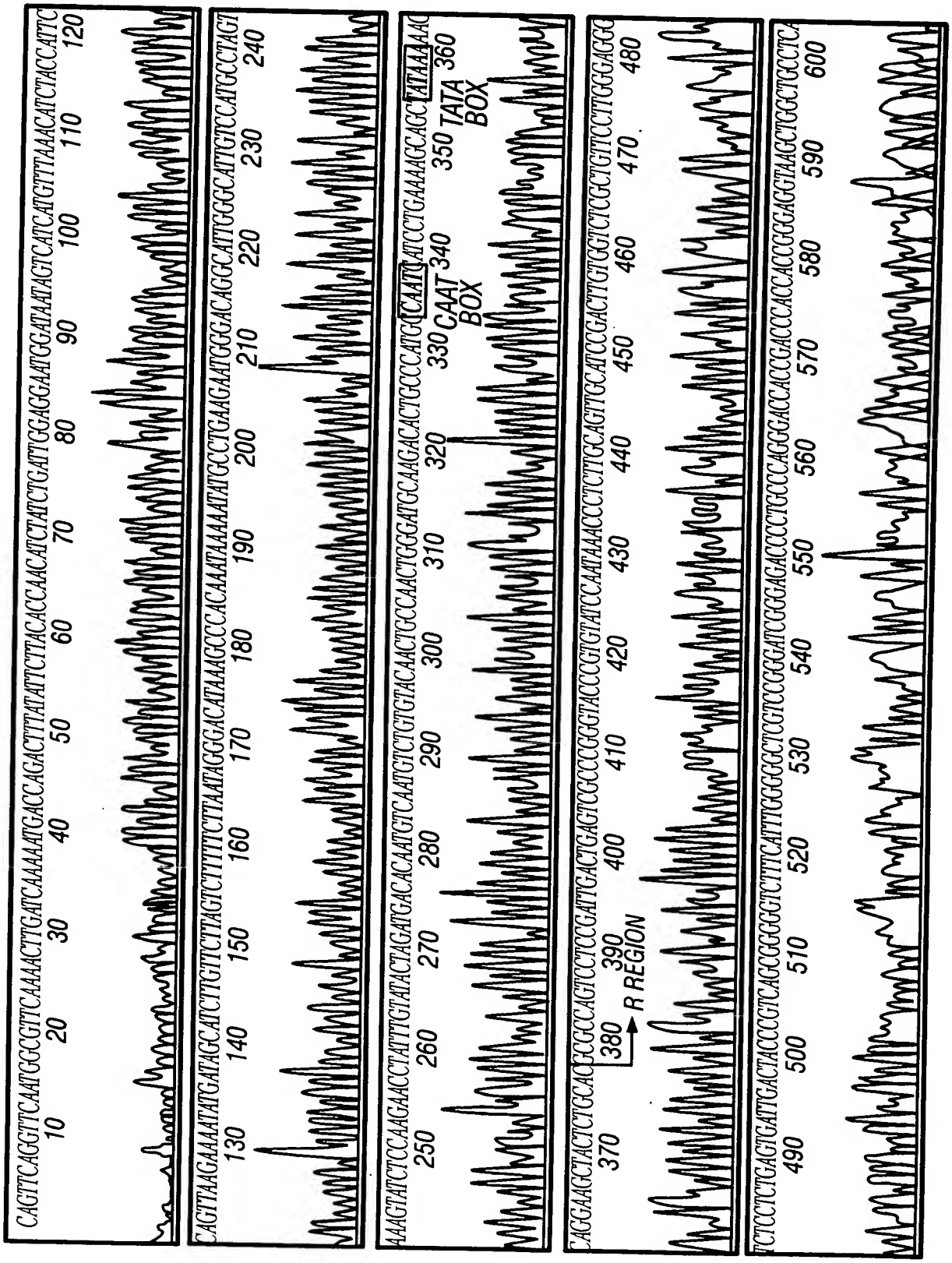
CAGTTCAGGTTCAATGGCGTTCAAAACTTGATCAAAAATGACCAGACTTTATATTCTTACACCAACATCTATCTGATTGGAAGGAATGGATATAGTCATCATGTTTAAACATCTACCATTC
10 20 30 40 50 60 70 80 90 100 110 120
CAGTTAAGAAAATATGATAGCATCTTGTTCTTAGTCTTTTTCTTAATAGGGACATAAAGCCCACAAATAAAATATGCCTGAAGAATGGGACAGGCATTGGGCATTGTCCATGCCTAGT
130 140 150 160 170 180 190 200 210 220 230 240
AAAGTATCTCCAAGAACCTATTTGTATACTAGATGACACAATGTCTGTGTACAACTGCCAACTGGGATGCAAGACACTGCCCATGCCAATGATCCTGAAAAGCAGCTATAAAAAC
250 260 270 280 290 300 310 320 330 340 350 360
CAAT BOX
TATA BOX
CAGGAAGCTACTCTGCACGCCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGG
370 380 390 400 410 420 430 440 450 460 470 480
R REGION
TCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCTGCCTC
490 500 510 520 530 540 550 560 570 580 590 600

FIG. 9B

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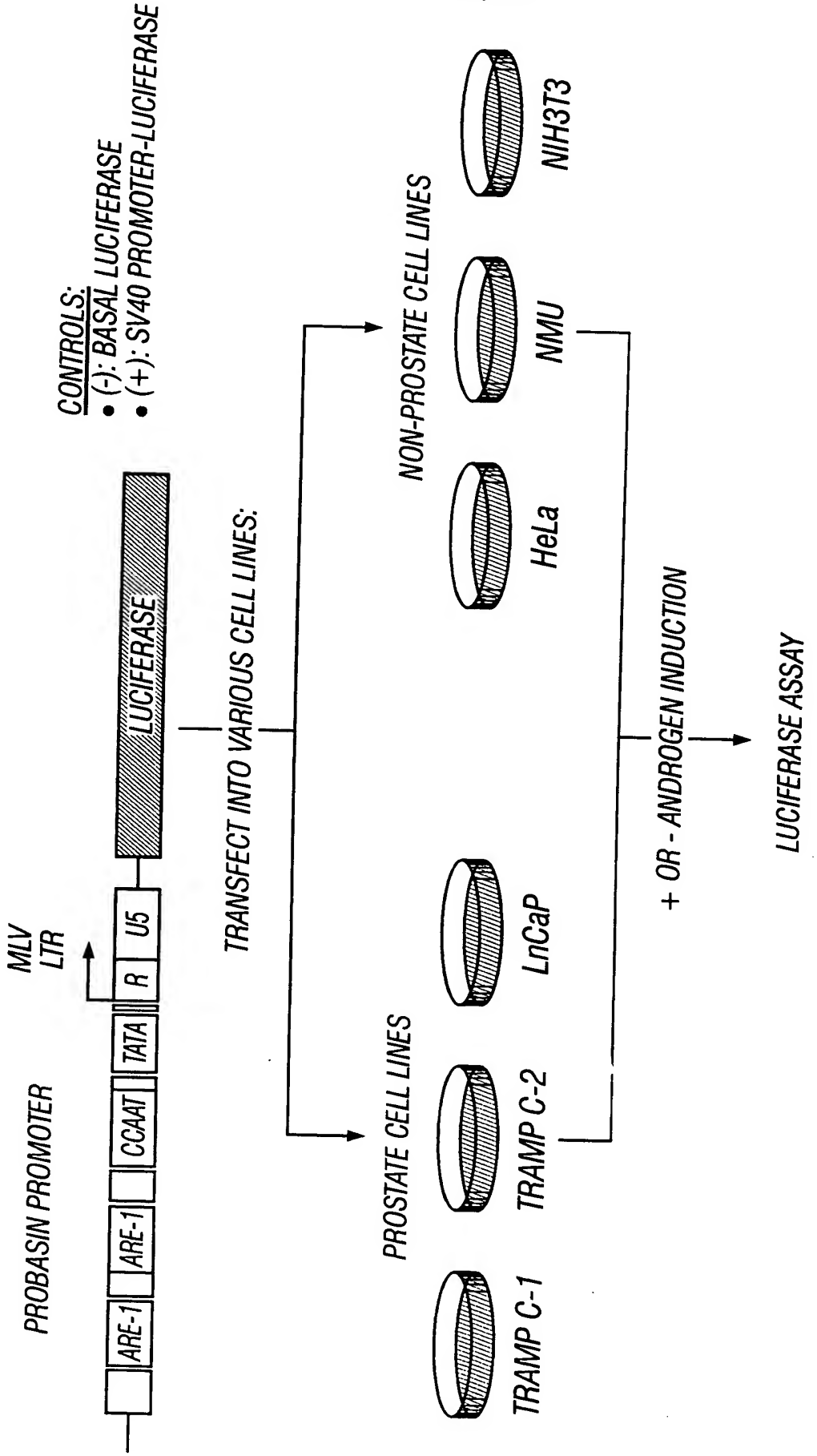
CONTROLS:
• (-): BASAL LUCIFERASE
• (+): SV40 PROMOTER-LUCIFERASE
PROBASIN PROMOTER
ARE-1
ARE-1
CCAAT
TATA
MLV LTR
R
U5
LUCIFERASE
TRANSFECT INTO VARIOUS CELL LINES:
PROSTATE CELL LINES
TRAMP C-1
TRAMP C-2
LnCaP
NON-PROSTATE CELL LINES
HeLa
NMU
NIH3T3
+ OR - ANDROGEN INDUCTION
LUCIFERASE ASSAY

FIG. 10

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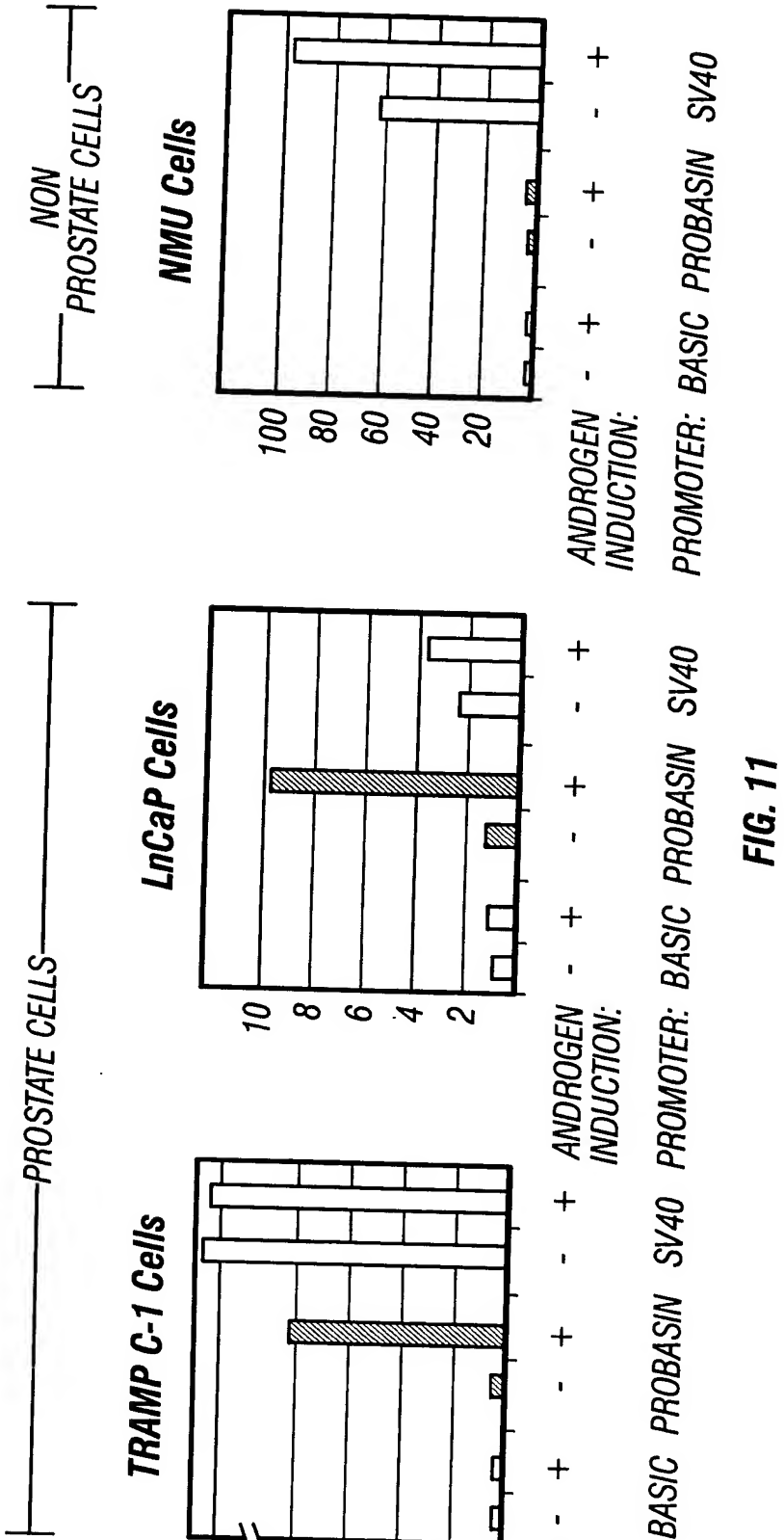
NON PROSTATE CELLS
NMU Cells
100
80
60
40
20
ANDROGEN INDUCTION: - + - + - +
PROMOTER: BASIC PROBASIN SV40
PROSTATE CELLS
LnCaP Cells
10
8
6
4
2
ANDROGEN INDUCTION: - + - + - +
PROMOTER: BASIC PROBASIN SV40
TRAMP C-1 Cells
40
20
15
10
5
ANDROGEN INDUCTION: - + - + - +
PROMOTER: BASIC PROBASIN SV40

FIG. 11

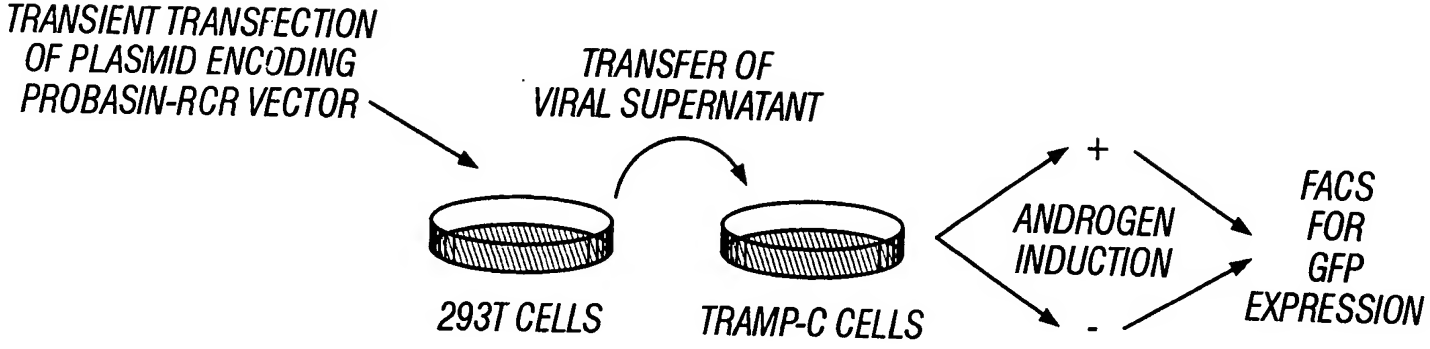
TRANSIENT TRANSFECTION
OF PLASMID ENCODING
PROBASIN-RCR VECTOR
TRANSFER OF
VIRAL SUPERNATANT
293T CELLS
TRAMP-C CELLS
+
ANDROGEN
INDUCTION
-
FACS
FOR
GFP
EXPRESSION

FIG. 12

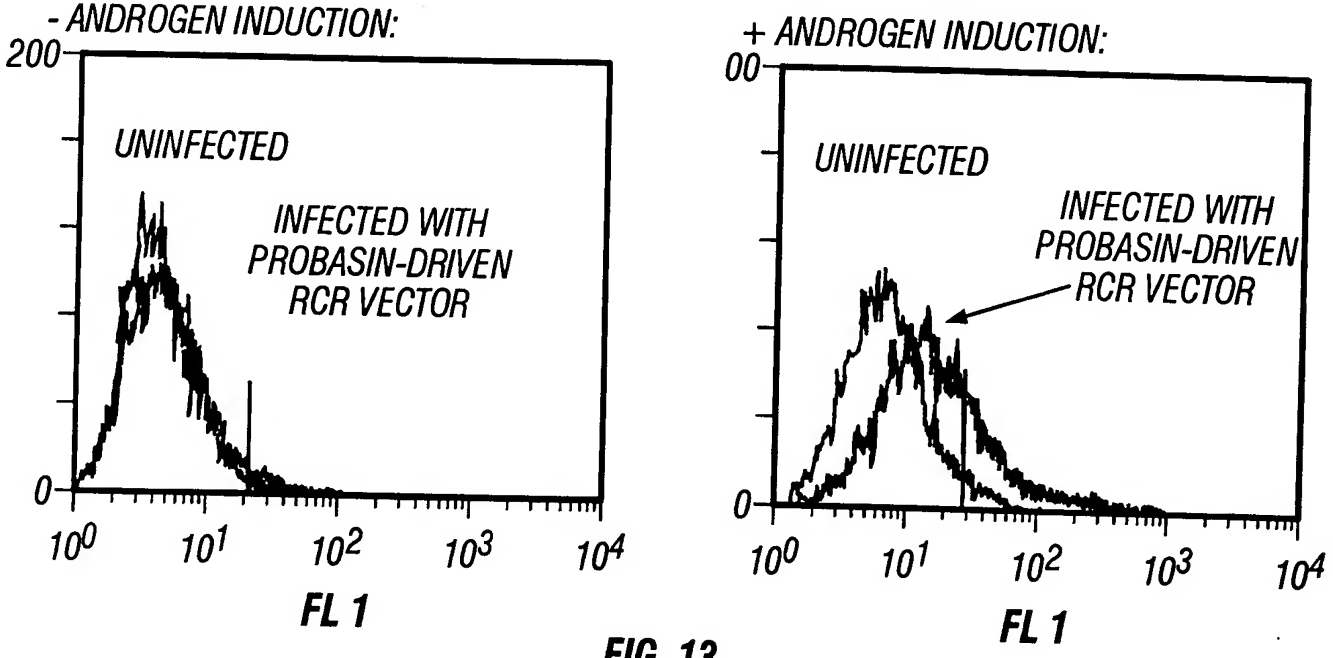
- ANDROGEN INDUCTION:
200
UNINFECTED
INFECTED WITH
PROBASIN-DRIVEN
RCR VECTOR
0
10^0
10^1
10^2
10^3
10^4
FL 1
+ ANDROGEN INDUCTION:
00
UNINFECTED
INFECTED WITH
PROBASIN-DRIVEN
RCR VECTOR
0
10^0
10^1
10^2
10^3
10^4
FL 1

FIG. 13

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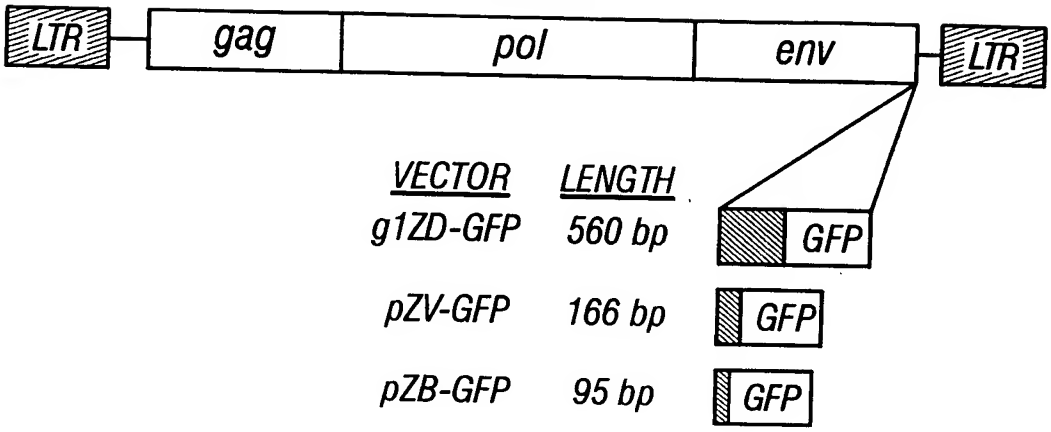
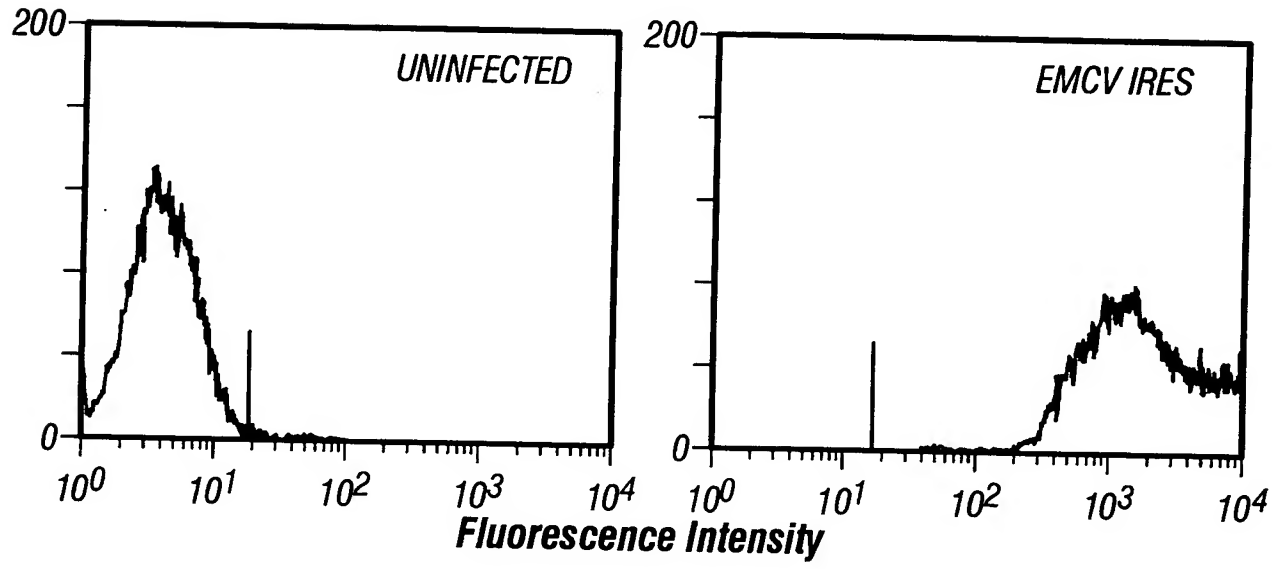


FIG. 14



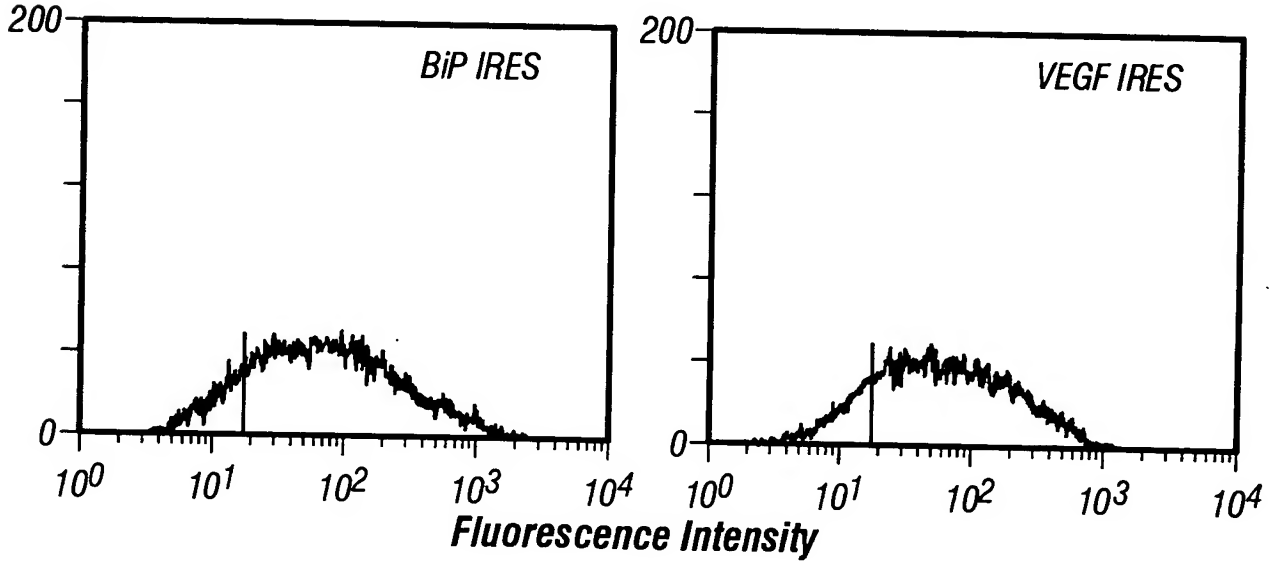


FIG. 15

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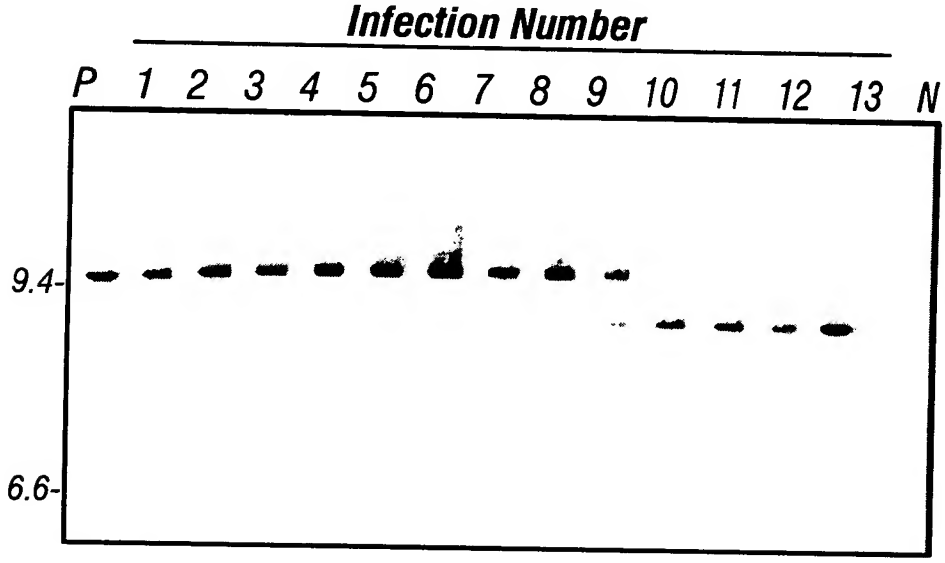

FIG. 16A

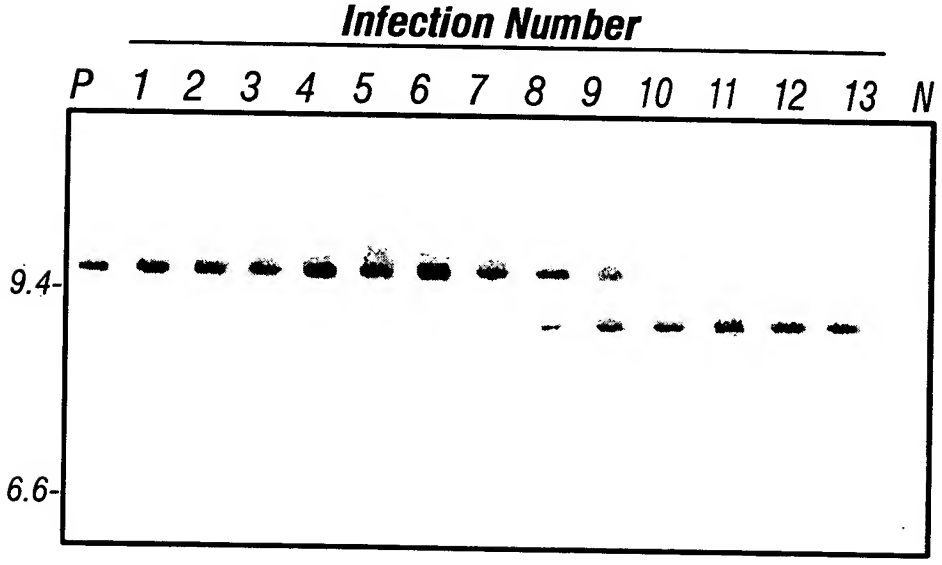

FIG. 16B

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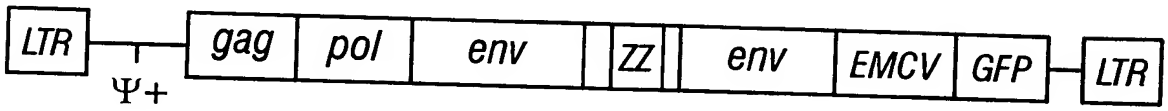
ZE-GFP



ZV-A-GFP

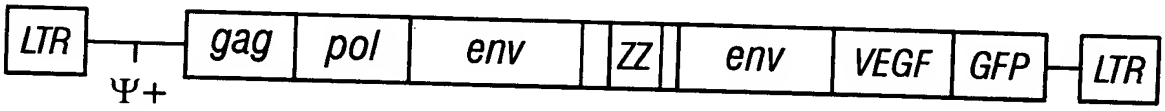
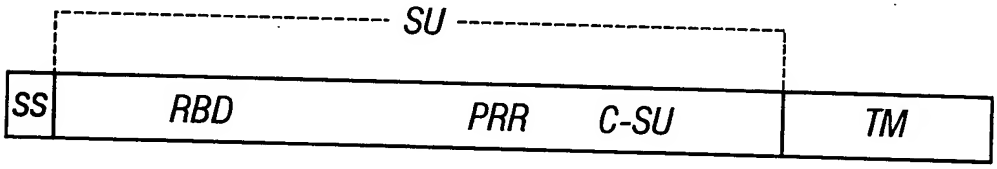
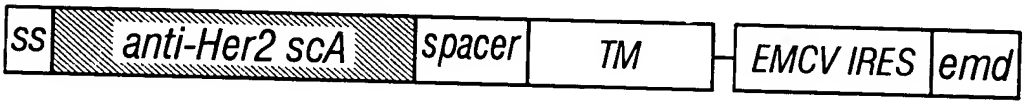


FIG. 17

WILD TYPE MLV ENVELOPE



ZDAH-emd



ZBAH-emd

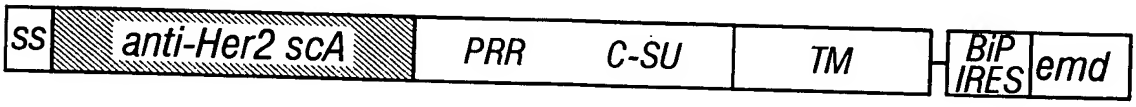


FIG. 18

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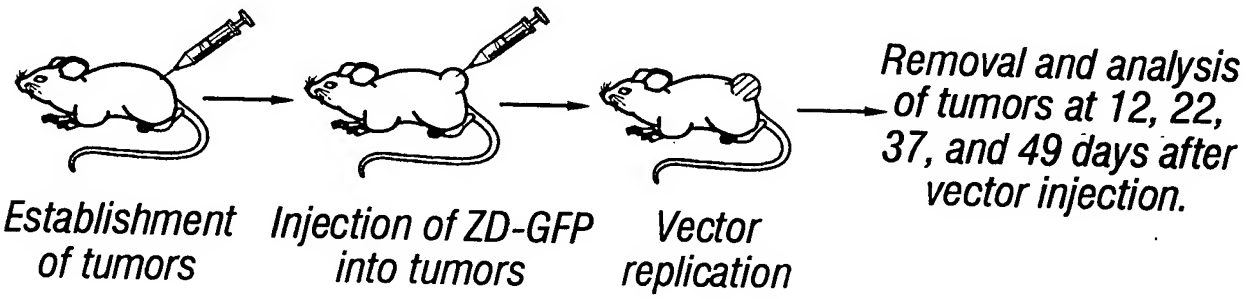

FIG. 19

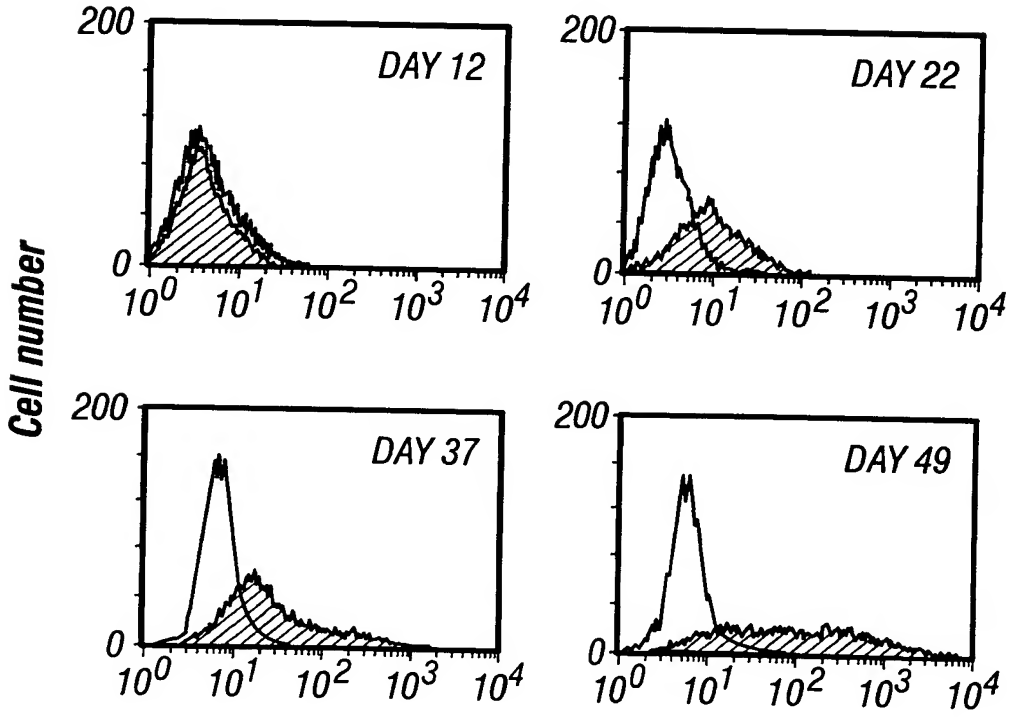

FIG. 20

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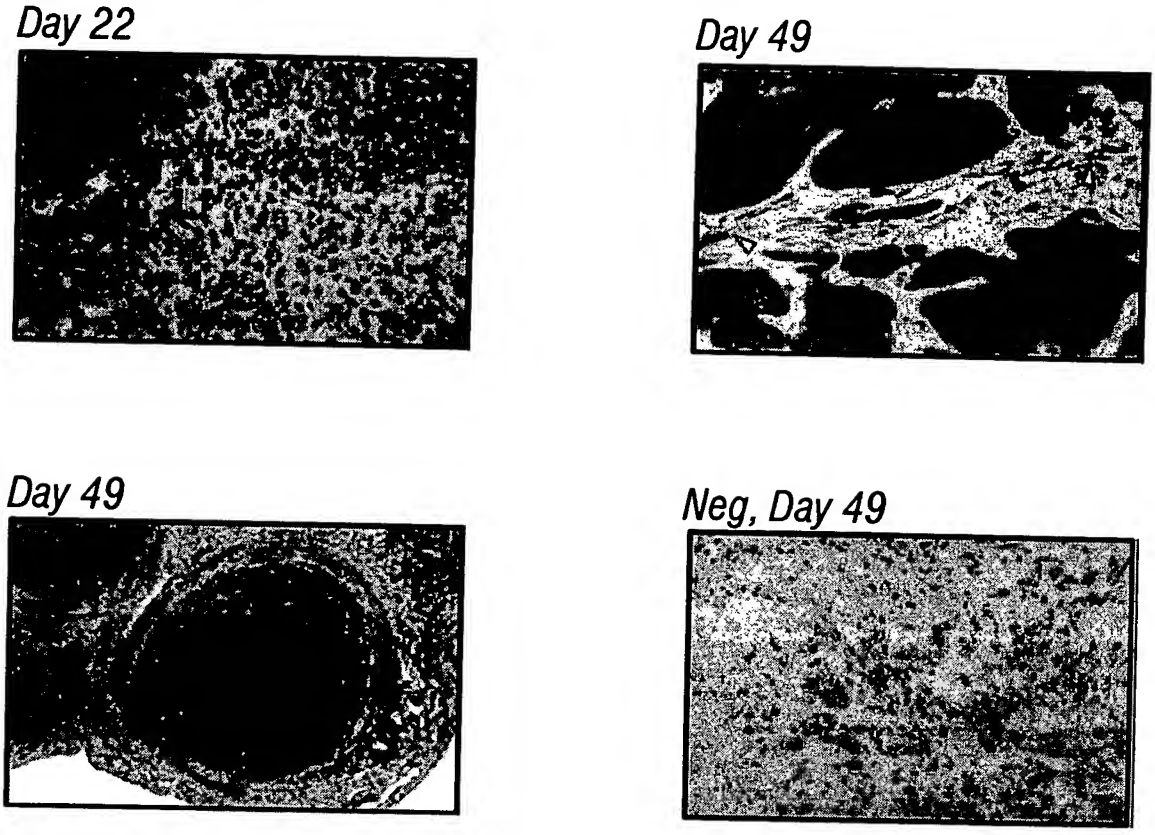

FIG. 21

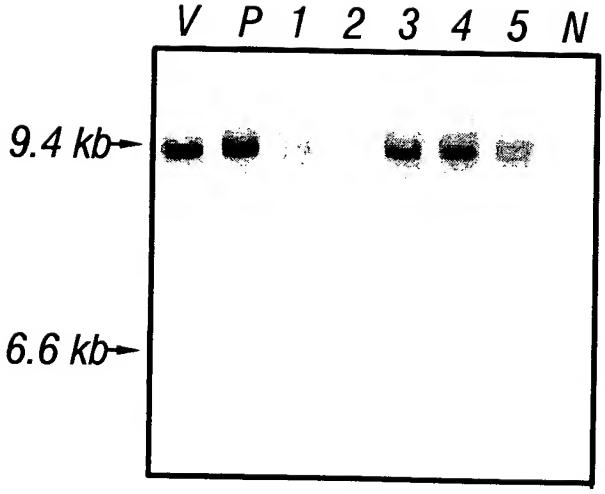

FIG. 22A

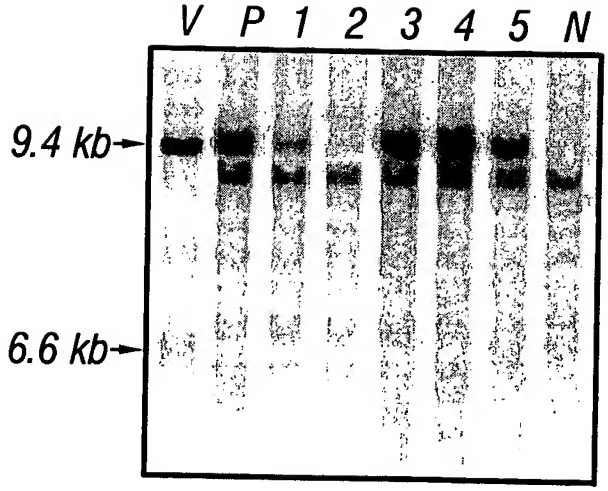

FIG. 22B

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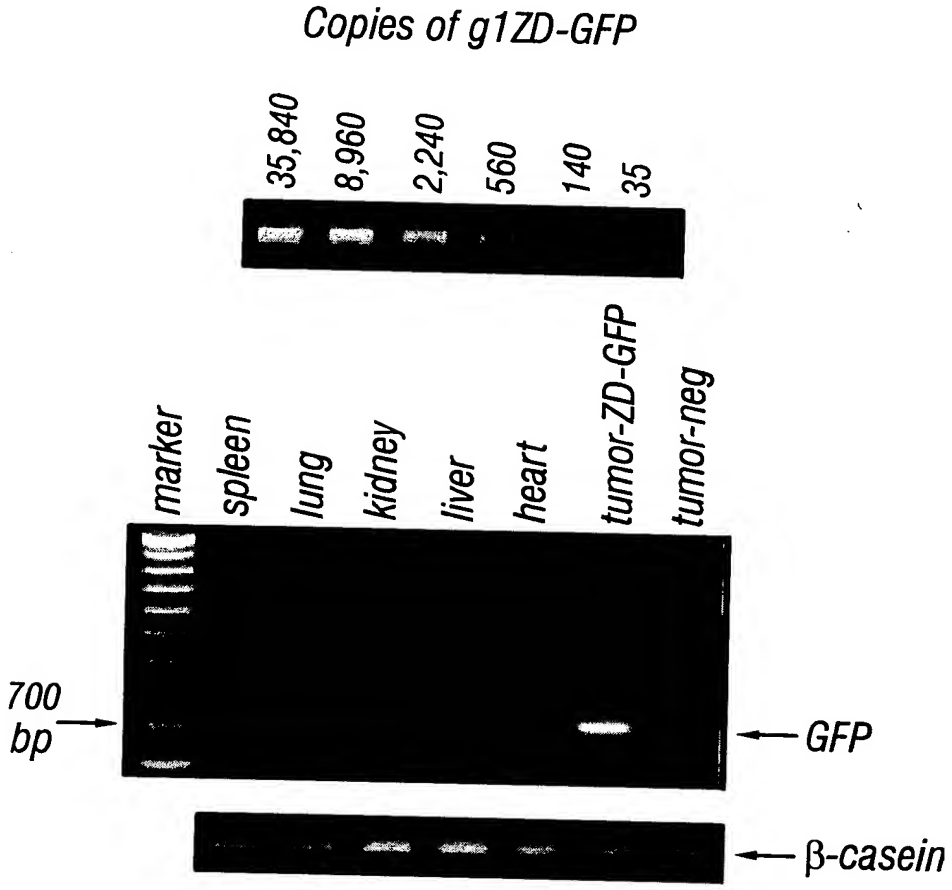
Copies of g1ZD-GFP
35,840
8,960
2,240
560
140
35
marker
spleen
lung
kidney
liver
heart
tumor-ZD-GFP
tumor-neg
700 bp
GFP
β-casein

FIG. 23

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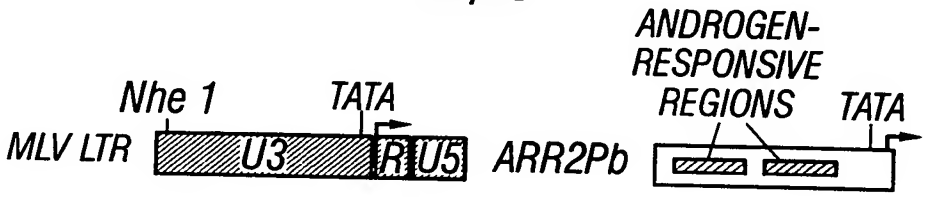

FIG. 24A

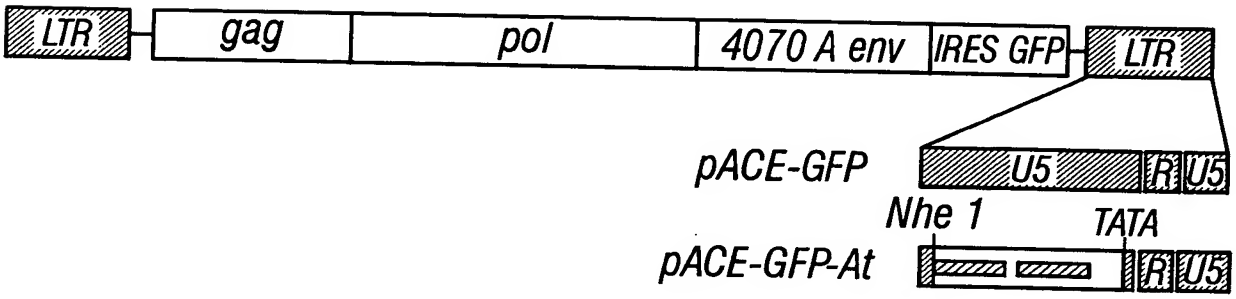

FIG. 24B

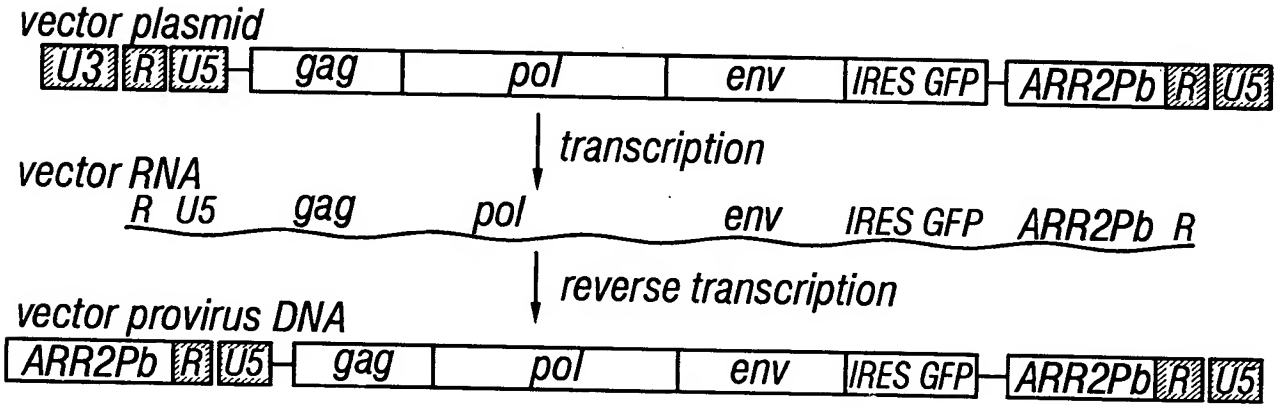

FIG. 25

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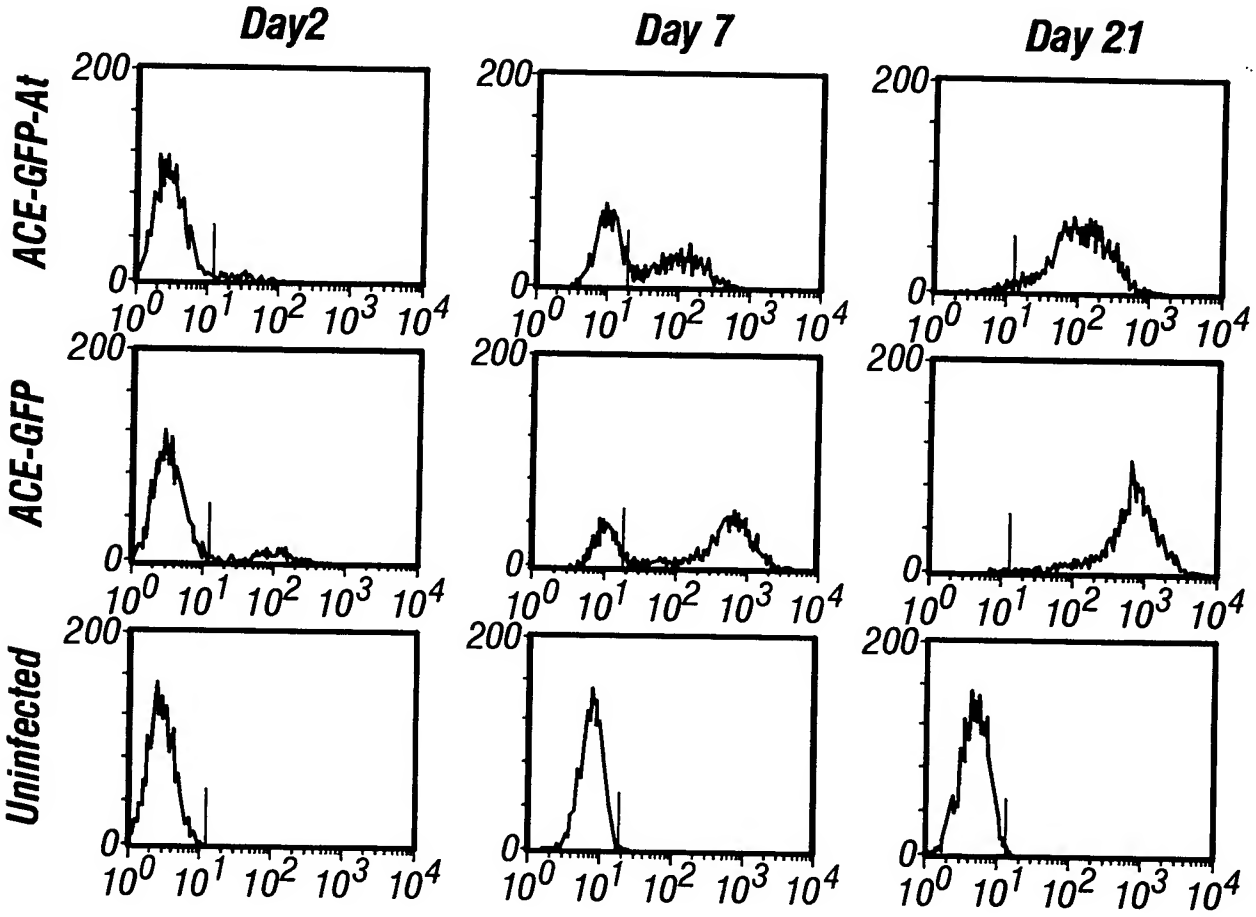
Day2
Day 7
Day 21
ACE-GFP-At
ACE-GFP
Uninfected
200
0
10^0 10^1 10^2 10^3 10^4

FIG. 26

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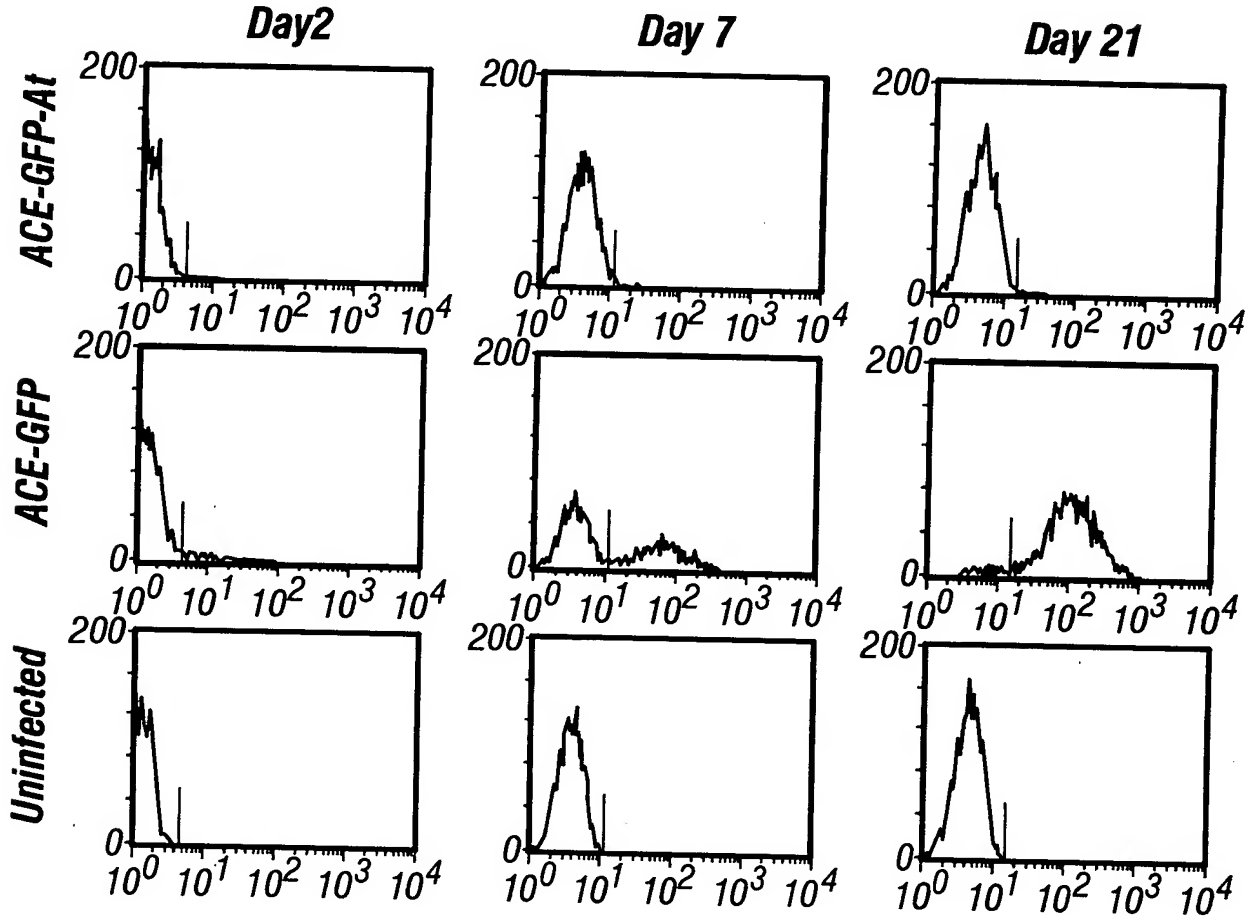
Day2
Day 7
Day 21
ACE-GFP-At
ACE-GFP
Uninfected
200
0
10^0 10^1 10^2 10^3 10^4

FIG. 27

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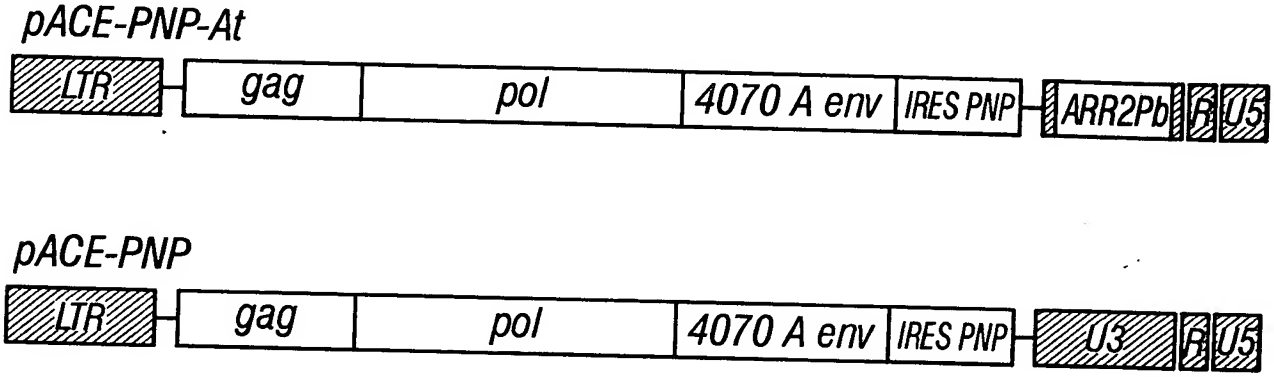

FIG. 28A

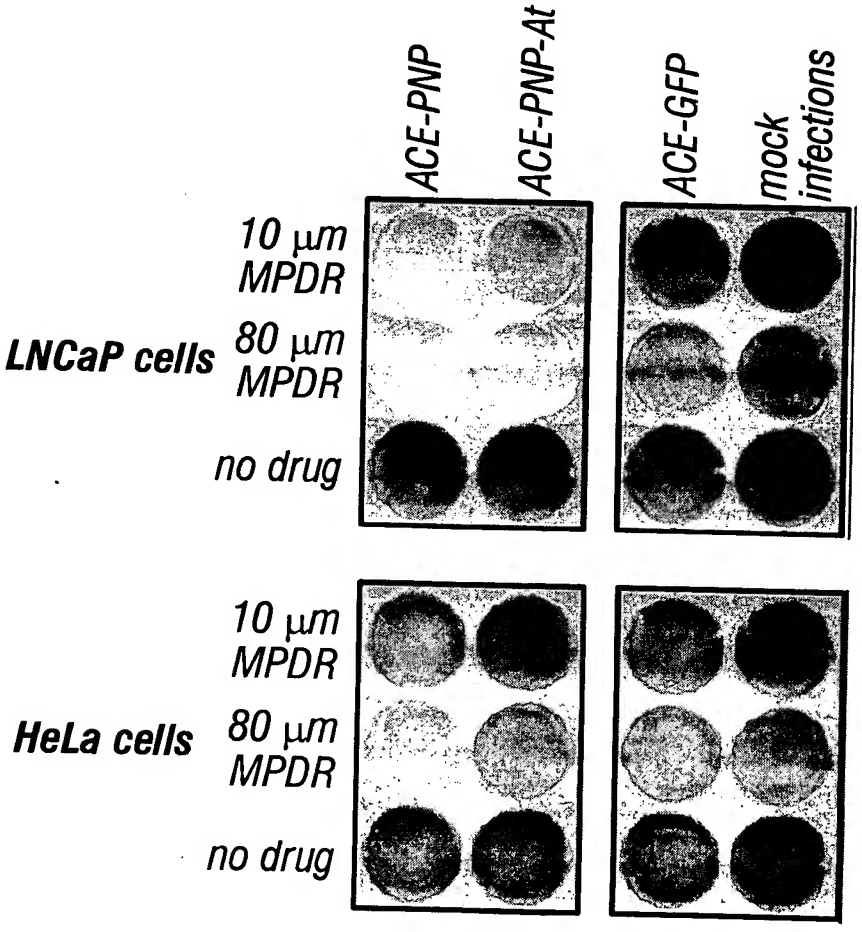

FIG. 28B